Journal of Steroid Biochemistry, 1977, Vol. 8, pp. 1159–1163. Pergamon Press. Printed in Great Britain

AMNIOTIC FLUID CORTISOL DURING GESTATION AND ITS RELATION TO FETAL LUNG MATURATION

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(*Received* 17 *March* 1977)

SUMMARY

The concentration of unconjugated cortisol was determined with a specific radioimmunoassay in amniotic fluid samples collected by amniocentesis in uncomplicated pregnancies and in pregnancies with mild complications. A slight increase in the mean levels was observed with advancing gestation, namely from 8.9 ng/ml in the group of 13–24 weeks to 13.8 ng/ml in the group of 37–38 weeks of gestation. In the group of 39–42 weeks the mean cortisol level, 19.5 ng/ml, was, however, significantly higher, due perhaps to an increase in activity of fetal adrenals prior to term.

In an attempt to study the possible increase in activity of fetal adrenal cortisol secretion associated with the process of fetal lung maturation, the well-known markers of fetal lung maturity, lecithin–sphingomyelin ratio (L/S) and lecithin bound palmitic acid (LBP) were determined in the same amniotic fluid samples. These showed that in most cases the lung maturation had taken place in 33–38 weeks of gestation. During this period there was no evidence of any raised mean cortisol levels in the amniotic fluid. In 12 paired follow-up amniotic fluid samples collected in 35–38 weeks with 1–2 week intervals, a rise of L/S and LBP indicated that fetal lung maturation was taking place. In 10 of these cases a concomitant rise of cortisol concentration was found.

INTRODUCTION

There is some evidence, both *in vitro* [1] and *in vivo* [2, 3] that human fetal adrenals secrete cortisol throughout the latter half of gestation. Cortisol is excreted from the fetal circulation partly via the fetal urine into the amniotic fluid. On the other hand, maternal cortisol is also transferred into the fetal compartment. During the transfer across the placenta cortisol is, however, extensively metabolised into cortisone [4]. It is probable, therefore, that amniotic fluid cortisol concentration reflects more closely fetal than maternal adrenal function. Evidence of this has also been obtained recently; Murphy *et al.*[5] found a better correlation of amniotic fluid cortisol level to the fetal than to the maternal blood cortisol level.

The biological significance of fetal adrenal function in human pregnancy is at present obscure. It has been suggested that during the latter half of pregnancy fetal cortisol initiates the synthesis of fetal pulmonary surfactant [6]. And at term pregnancy, fetal cortisol may play a role in the initiation of labour [7].

The relationship between amniotic fluid cortisol concentration and the duration of pregnancy has been studied by Murphy *et al.*[5]. Fencl *et al.*[8] and Tan *et al.*[9]. The two last-mentioned authors also found a positive correlation between amniotic fluid lecithin–sphingomyelin ratio (L/S) and cortisol level. This contrasts the findings of Sivakumaran *et al.*[10] who did not find any good correlation between amniotic fluid cortisol level and L/S. Recently poor correlation has also been reported between the levels of amniotic fluid cortisol and palmitic acid, another marker of fetal lung maturation [11]. The available data on amniotic fluid cortisol concentration in the 30–38 week period of gestation, when the fetal lung maturation takes place, is insufficient. No simultaneous follow-up of amniotic fluid cortisol level and L/S seems to have been performed during this period. Here we wish to report results of amniotic fluid cortisol determinations with a specific radioimmunoassay. The process of fetal lung maturation was followed up by determining L/S and lecithin bound palmitic acid concentration (LBP) in the same amniotic fluid samples.

EXPERIMENTAL

Subjects

Amniotic fluid samples were collected by amniocentesis in 76 pregnancies. In 23 of these, a repeated amniocentesis was performed after a 1–2 week interval. Twenty nine pregnancies were uncomplicated. In 9 of these, amniotic fluid was collected before intra-amniotic induction of abortion with prostaglandin. Eighteen pregnancies were complicated by Rh-isoimmunization, and 17 women had diabetes; 4 of these had latent, 11 had White's class B and 2 class C diabetes. Ten pregnancies were complicated by mild hypertension or by mild toxaemia. Three pregnant women had hepatosis of pregnancy. Cases with signs

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of fetal distress, e.g. fetal growth retardation or a pathological oxytocin challenge test, were excluded from this study. None of the mothers studied were in labour when the samples were collected.

Methods

Immediately after collection, amniotic fluid samples were centrifuged 3000 rev./min for about 5 min, and the supernatant was stored at −18°C until analyzed.

The amniotic fluid lecithin–sphingomyelin ratio (L/S) was determined essentially as described by Gluck *et al.*[12]. The cold acetone precipitation step was not used. Lecithin and sphingomyelin spots were visualized with iodine vapour after t.l.c. and compared with a specific L/S of the reference standard mixtures chromatographed simultaneously.

For determination of *lecithin bound palmitic acid* (*LBP*), an equal amount of methanol was added to 0.25–1.0 ml samples of amniotic fluid and phospholipids were extracted with chloroform. The extract was subjected to t.l.c. in chloroform:methanol:ammonia:water (130:50:4:4, v/v). The lecithin spot was visualized with iodine vapour, scraped off into a test tube and subjected to saponification with 0.5 ml of 0.5 N methanolic sodium hydroxide 5 min at 70°C, followed by esterification with 0.5 ml of BF_3-methanol 5 min at 70°C [13]. One ml of saturated NaCl solution was added, and fatty acid methyl esters were extracted with hexane. Quantitation of palmitic acid methyl ester was carried out by gas chromatography with an OV-101 glass capillary column. A suitable amount of nonadecanoic acid methyl ester was added to the sample as an internal standard for gas chromatographic quantitation, which was carried out by comparing the peak height response of the palmitic acid methyl ester with that of the internal standard. Recoveries of palmitic acid when a reference dipalmitoyl lecithin was added to amniotic fluid samples varied from 84 to 89%.

Amniotic fluid unconjugated cortisol was measured in 1–2 ml samples with a specific radioimmunoassay developed for serum and urine cortisol determinations by Apter *et al.*[14]. The specificity of the determinations was based on a purification step with Lipidex-5000 chromatography [14] and, furthermore, on a specific antiserum which had a low affinity to other steroids *e.g.* progesterone and corticosterone [14], known to be present in amniotic fluid. Recoveries or cortisol added to amniotic fluid samples varied from 90 to 108%.

RESULTS

Cortisol concentrations in amniotic fluid samples in relation to the duration of gestation can be seen in Fig. 1. An increasing tendency was observed in cortisol values with advancing gestation. In the full-term group of 39–42 weeks the mean cortisol concentration was 19.5 ng/ml, which was considerably higher than the concentration found in the other groups in earlier gestation. The comparison of this value with the corresponding value in the group of 37–38 weeks of gestation (13.8 ng/ml), for example, showed that the difference was statistically significant ($p < 0.02$) according to Student's *t*-test.

Results of L/S and LBP determinations in the amniotic fluid samples can be seen in Figs. 2 and 3, respectively. An increase in the proportion of samples with raised LBP (>13 μg/ml) was observed when the gestation was advanced from 33 to 38 weeks

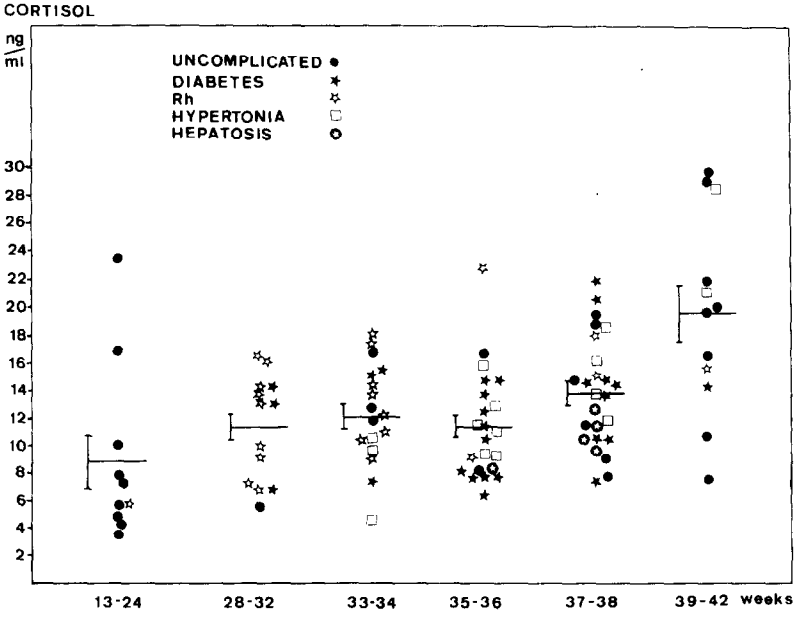

Fig. 1. Concentration of cortisol in the amniotic fluid in relation to advancing gestation. The transverse lines are mean levels and the bars standard errors of the means.

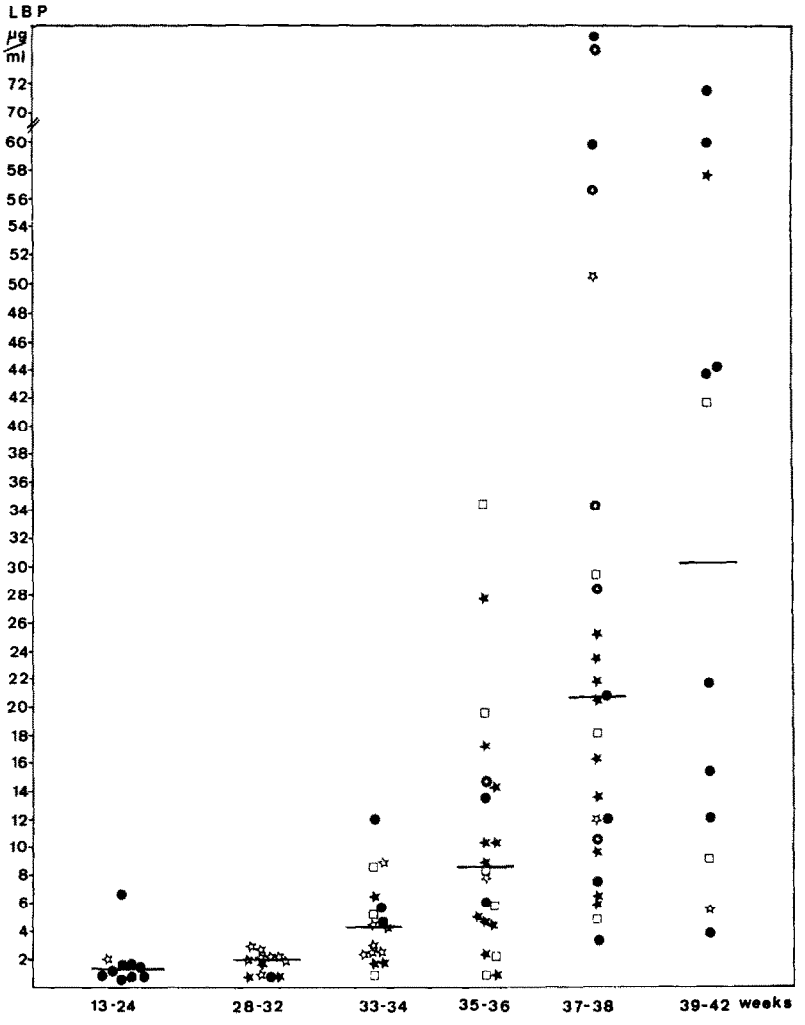

Fig. 2. Lecithin bound palmitic acid concentration (LBP) in the amniotic fluid samples. The transverse lines represent the medians of the groups. The symbols for various groups are as in Fig. 1.

(Fig. 2); the proportion of samples with L/S 1.5–2 and >2 increased simultaneously (Fig. 3). Amniotic fluid cortisol levels were, however, not elevated nor could any differences be found in the mean cortisol levels between the various groups in this period of gestation (Fig. 1).

The series in 28–40 weeks of gestation was divided into three goups according to L/S, and the groups were compared for cortisol concentration (Fig. 4). No significant differences could be found in the mean cortisol levels.

In order to eliminate an interindividual variation in amniotic fluid cortisol levels, paired follow-up samples were collected in 23 cases within 1–2 week intervals (Table 1). In 11 sample pairs, with the first sample taken at 29–34 weeks of gestation, no statistically significant change was found in either LBP or cortisol levels. In the 12 sample pairs where the collection started at 35 weeks or later, a significant increase of LBP ($p < 0.01$) and also a change of L/S to 1.5–2 or >2 were observed in all cases. A rise of cortisol concentration was observed in 10 of these 12 sample pairs and a fall in two. The *t*-test for paired observations showed a significant rise in the cortisol level in these 12 sample pairs ($p < 0.05$).

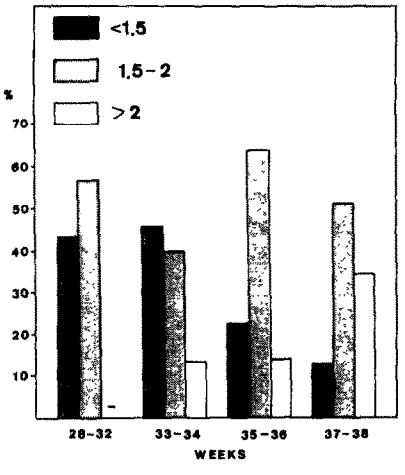

Fig. 3. Results of L/S determinations in relation to advancing gestation.

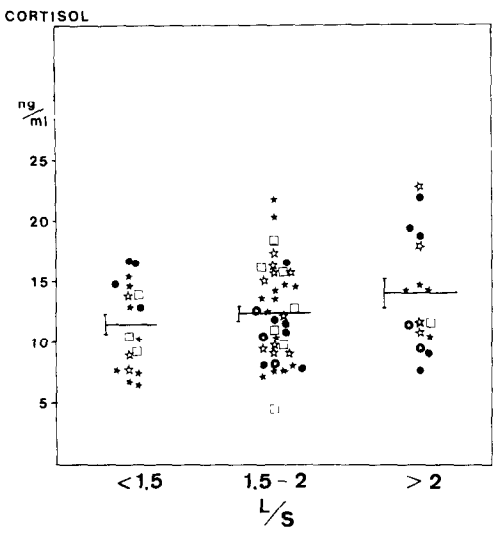

Fig. 4. Relationship of amniotic fluid cortisol concentration to L/S in the amniotic fluid samples. The symbols for various groups are as in Fig. 1.

DISCUSSION

Since the purpose of this study was to establish if there is any correlation between amniotic fluid cortisol and the process of lung maturation, most amniotic fluid samples were taken at 30–38 weeks of gestation when the fetal lung maturation takes place. It was not possible to obtain a sufficient number of samples during this period of gestation in uncomplicated pregnancies. Therefore, mild pregnancy complications were included, in which no evidence of a disturbance to the fetal well-being could be found. The concentrations of unconjugated cortisol in the amniotic fluid were found to be in agreement with the findings of Murphy *et al*[5] and Tan *et al.*[9], but much lower than those found by Fencl *et al.*[8], who measured both unconjugated and conjugated cortisol.

There is some evidence that corticosteroids may initiate the process of fetal lung maturation. Infusion of fetal lambs with glucocorticoids resulted in accelerated lung maturation [15]. The presence of a specific receptor for corticosteroids has been demonstrated in the fetal lung tissue [16, 17]. Cortisol has been found to promote the growth of fetal lung tissue [18] and to initiate the synthesis of the pulmonary surfactant in the preparations of fetal lung tissue [19]. Furthermore, corticosteroid administration to the mother has been reported to diminish the risk of the newborn developing a respiratory distress syndrome [20]. If there is an increase in the fetal cortisol secretion at the time of fetal lung maturation, this might reflect an elevated amniotic fluid cortisol level during this period of gestation. When the synthesis of the fetal lung surfactant has begun, dipalmitoyl lecithin appears in the amniotic fluid, and this can be detected by means of L/S [12] or by determining the amniotic fluid palmitic acid level [21, 22]. Amniotic fluid contains both free [23] and lecithin bound palmitic acid; the latter was measured in the present study in order to obtain more specific information concerning dipalmitoyl lecithin synthesis. Because interindividual variation in LBP and cortisol levels was considerable, a follow-up of L/S, LBP and cortisol in paired amniotic fluid samples collected with advancing gestation was performed. In 12 cases at 35–38 weeks of gestation (Table 1) a significant rise of LBP and L/S showed that fetal lung maturation was taking place.

Table 1. A follow-up of the concentration of cortisol, L/S and LBP in paired amniotic fluid samples

Case No.	Week	L/S	LBP μg/ml	Cortisol ng/ml	Diagnosis
1	29	1.5	2.1	9.1	Rh-immunization
	31	1.0	2.5	7.8	
2	30	ND	2.1	14.1	Rh-immunization
	32	ND	2.9	16.1	
3	31	ND	1.9	13.0	Rh-immunization
	33	ND	1.9	10.3	
4	31	1.5	0.8	14.2	Diabetes
	33	1.0	1.7	15.0	
5	32	1.5	ND	16.3	Rh-immunization
	34	>2	ND	18.0	
6	33	1	1.0	10.6	Hypertonia
	35	1	0.9	9.2	
7	33	1	2.3	13.8	Rh-immunization
	34	1.5	4.4	17.4	
8	33	1.5	5.3	9.7	Toxaemia
	34	2	8.6	4.6	
9	33	1	1.7	7.4	Diabetes
	35	1	1.0	6.3	
10	33	1.5	12.0	11.9	Uncomplicated
	34	>2	8.9	10.9	
11	34	1.5	16.3	15.6	Diabetes
	36	2	10.2	14.7	
12	35	1	0.9	9.2	Hypertonia
	36	1.5	2.1	11.0	
13	35	1.5	9.0	7.6	Diabetes
	36	2	14.2	8.1	
14	36	1.5	5.0	13.7	Diabetes
	37	2	13.7	14.6	
15	36	1.5	27.7	12.3	Hypertonia
	37	>2	75.5	18.7	
16	36	1	10.3	14.7	Diabetes
	37	2	20.4	13.2	
17	36	1.5	5.6	10.9	Diabetes
	37	>2	9.0	7.4	
18	36	2	14.7	8.2	Hepatosis of
	37	>2	56.4	9.5	pregnancy
19	36	1.5	4.4	7.7	Latent diabetes
	37	1.5	6.3	21.9	
20	37	1	5.9	10.3	Diabetes
	38	2	9.7	13.6	
21	37	1	4.8	13.8	Hypertonia
	38	2	29.4	16.1	
22	37	1.5	10.5	10.4	Hepatosis of
	38	2	28.2	12.4	pregnancy
23	38	2	25.2	7.3	Latent diabetes
	39	>2	57.4	14.2	

In 10 of these a concomitant rise of cortisol concentration was observed, which may be due to an increase in fetal cortisol secretion associated with the process of fetal lung maturation.

When the groups at 33–38 weeks of gestation were compared, it was found that the proportion of cases with the mature L/S (>2) and those with raised levels of LBP increased sharply with increasing gestational age indicating advancing maturation of the fetal lungs. We were not, however, able to demonstrate any increase in the mean cortisol levels in the amniotic fluid during this period of gestation (Fig. 1). No significant correlation could be found between L/S and amniotic fluid cortisol, which contrasts with the findings of Fencl *et al.*[8] and Tan *et al.*[9]. This might be due to differences in the materials used. In the present study the period when amniotic fluid samples were collected was more restricted and excluded both early pregnancies as well as cases near term. According to the present results, the value of a single amniotic fluid cortisol determination in the predictive assessment of the risk of the fetus developing RDS suggested by Fencl *et al.*[8] is highly questionable. The group of 39–42 weeks of gestation had a significantly higher amniotic fluid cortisol level than the groups of earlier gestation (Fig. 1). This agrees with the findings of Murphy *et al.*[5]. At term pregnancy, maternal and fetal blood levels or cortisol have also been found to be elevated [24, 25]. This finding has been explained as being a result of the stress of labour, since cortisol determinations were performed in association with labour [24]. None of our subjects, however, was in labour. No sudden rise near term in the maternal plasma cortisol levels was found by Brien and Dalrymple[26], although the maternal levels rose steadily throughout pregnancy. The present results suggest that there is an increase in the activity of fetal adrenals prior to term. In animals the rise of the fetal cortisol secreting activity is known to initiate labour [27], and this factor has also been suggested as playing a role in the initiating of human labour [7]. An increase in the fetal cortisol secretion prior to term may also have an important biological significance in initiating the maturation of enzyme systems necessary for the extrauterine life of the fetus as suggested by Thorburn *et al.*[28].

Pasqualini *et al.*[29] injected labeled cortisol into the intact fetoplacental circulation of the midgestation fetuses and found it largely converted to cortisone and other metabolites in various fetal tissues. Thus, cortisol secreted by the fetal adrenals is partly metabolized in the fetus before excretion into the amniotic fluid. This may limit the usefulness of amniotic fluid cortisol concentration as a marker of fetal adrenal function. Further studies on the levels of the metabolites of cortisol in the amniotic fluid may give additional information on this subject.

Acknowledgements—The skillful technical assistance of Miss Marjatta Vallas is gratefully acknowledged. This work was supported by a grant from the National Research Council for Medical Sciences, Finland.

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